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The Pigment Glands of Cottonseed */

I. Behavior of the Glands Toward Organic Solvents

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THE current expansion of the oilseed processing industry has led to an increased use of solvent extraction methods. In view of the recent development of many improvements in solvent extraction processes (10), it might be expected that their extension to the processing of cottonseed would not be difficult and would involve primarily an engineering problem. Examination of the literature on the subject (7,11,12,13) indicates, however, that solvent extraction of cottonseed presents many problems, chief among which is that involving the complex pigment system which is present in cottonseed.

Cottonseed is very much more highly pigmented than most other oilseeds. Moreover, the amount of pigmented material extracted from the seed is a function of the type of solvent used. The behavior of the cottonseed pigments during solvent extraction of cottonseed as well as their stability in the seed is not readily explained on the basis of the solubilities of the isolated pigments. Petroleum ether extracts very little of the yellow cottonseed pigment, gossypol, despite the fact that gossypol is readily soluble in mixtures of hydrocarbons and cottonseed oil (15). Gossyfulvin, the recently isolated orange-colored isomer of gossypol, is soluble in pure hydrocarbon solvents but occurs in only relatively small amounts in petroleum ether extracts of cottonseed (2). Even the solvents which do extract the pigments effect complete extraction only after prolonged contact with the seed. For example, 24 hours' contact with diethyl ether or chloroform is the shortest time required for complete extraction of gossypol from finely ground cottonseed or cottonseed flakes (2).

The cottonseed pigments differ markedly from other plant pigments not only in their chemical nature but in their distribution in the seed (14,16,8). Whereas most plant pigments occur as discrete particles on the outside surface of the plants, dissolved in the cell sap, or concentrated in small cell inclusions known as plastids, the cottonseed pigments occur in walled-off cavities or glands many times larger than the surrounding parenchyma cells. It seemed not improbable that the segregation of the pigments in these specialized glands might account for their anomalous behavior in the seed, *i.e.*, the phenomenon of chemically unstable polyphenolic compounds remaining relatively unaltered in the presence of a reactive system, yet decomposing as soon as they are removed from their natural environment, as well as resisting extraction by solvents in which they are soluble. Accordingly, an investigation of the fundamental properties of the pigment glands was undertaken, which is being reported in part here.

Effect of Organic Liquids on the Glands in the Seed

The reaction of the pigment glands of upland cotton *(Gossypium hirsutum)* to a variety of organic liquids was determined by direct microscopic examination of freehand sections of the seed which had been immersed in the liquids for different lengths of time. It was found that thin sections of seeds became distorted and unrolled into long strands after immersion in organic liquids for even a short time so that determination of the condition of the glands by means of microscopic examination was difficult. Consequently, relatively thick sections were immersed in the liquids maintained in closed containers. The sections were then carefully transferred to slides for microscopic examination to determine the effect on the glands of contact with the solvents being tested.

The glands were found to be unaffected by even prolonged exposure to a large number of hydrocarbons, chlorinated hydrocarbons, and glycerides, such as petroleum ether, solvent naphtha, benzene, tetralin, decalin, mineral oil, 1,1,2-trichloroethylene, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, 1,1,2,2-tetrachloroethylene, carbon tetrachloride, and cottonseed oil. Immersion in solvents such as diethyl ether, chloroform, acetone, methyl ethyl ketone, and 1,4-dioxane for one to two hours caused the destruction of only a small percentage of the exposed glands in the seed sections, but after 24 hours practically all of the glands were broken and most of their contents had dissolved. It is evident that these liquids are solvents for the extraction of cottonseed pigments because of their ability to break the pigment glands, as well as to dissolve the pigments after the glands are broken.

The behavior of the pigment glands toward both inert and active solvents is illustrated in the photomicrographs of cottonseed shown in Figure 1, in which (a) represents a freshly cut transverse section, (b) the same section after immersion in Skellysolve F (petroleum ether) for 24 hours, (c) a second transverse section of the same seed, and (d) section (c) after immersion in diethyl ether for 24 hours. By comparison of Figure 1 (b) with 1 (a) it can be seen that exposure to Skellysolve F has little if any effect on the pigment glands. The occasional less highly pigmented pits observed in Figure 1 (b) probably represent the skeletons of the pigment glands which were cut during the sectioning of the seed and thereafter lost most of their contents by solution in the mixture of Skellysolve F and cottonseed oil with which they were in contact. On the other hand, as may be seen by comparison of Figure 1 (d) with Figure 1 (c), diethyl ether has attacked all of the exposed glands and dissolved most of their contents.

Microscopic examination of a series of samples of flaked cottonseed revealed the fact that the pigment glands were largely intact even in flakes having an average thickness less than that of the glands themselves. Also, very few broken glands were found in samples of seed which had been ground fine enough to pass a U. S. No. 80 sieve.

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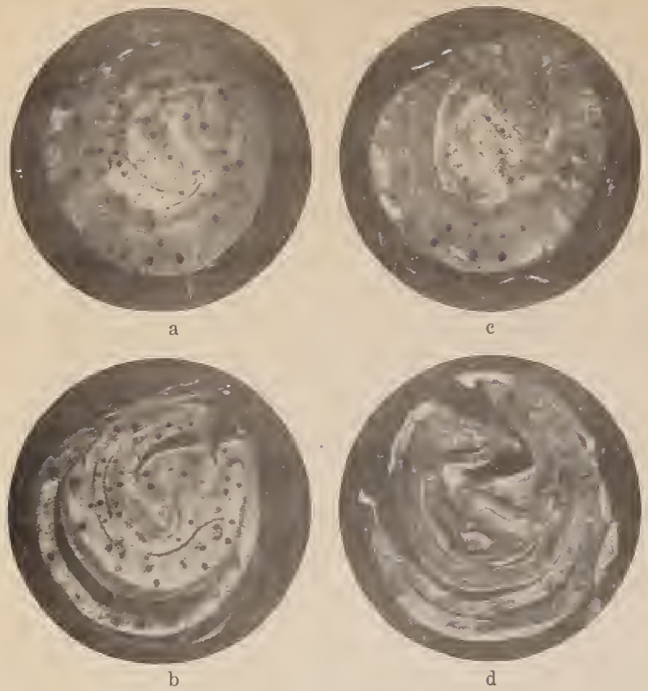

FIG. 1. Photomicrographs of transverse sections of cottonseed × 7 (a) freshly cut section, (b) section (a) after 24 hours immersion in Skellysolve F, (c) freshly cut section, (d) section (c) after 24 hours' immersion in diethyl ether.

In view of the high mechanical strength of the pigment glands it is safe to assume that after extraction of flaked or ground seed with various organic liquids any rupturing of these glands must necessarily result from the action of the solvents. Microscopic examination of extracted cottonseed flakes showed that the reaction of the pigment glands toward various solvents was analogous to their behavior in the transverse sections. A large number of intact pigment glands were observed in cottonseed flakes even after they had been subjected to exhaustive extraction with either petroleum ether or *n*-hexane. On the other hand, cottonseed flakes which had been extracted with diethyl ether contained few intact pigment glands. These few intact glands were inside of thick flakes where they were protected from the action of the solvent in contact with the surrounding tissue. Samples of cottonseed, which had been ground to pass through a U. S. No. 80 sieve prior to their treatment with such solvents as ether, chloroform, dioxane, and acetone contained only a negligible number of intact pigment glands after exposure to the action of these solvents, whereas samples of ground seeds which had been exhaustively extracted with petroleum ether or *n*-hexane contained practically as many intact pigment glands as were present in the original seed. Similarly, when suspensions of flaked cottonseed in inert liquids were violently agitated, *e.g.*, in a Waring Blendor, the flakes were broken into small fragments, but the pigment glands remained intact.

Obviously, the cottonseed pigment glands are very stable since they are not broken when the seed is rolled or ground and are largely unaffected when transverse sections, rolled flakes, or ground seed are exposed to contact with a variety of inert organic liquids. Even active organic solvents attack the pigment glands only upon prolonged contact. Observations of the effect of various solvents on the exposed pigment glands in transverse sections and rolled flakes are summarized in Table 1.

The microscopic observations of cottonseed indicate clearly that the localization of the cottonseed pigments in glands is largely responsible for their stability in the seed. Only a small proportion of these very resistant pigment glands are broken when cottonseed is ground or flaked, and the intact glands in transverse sections, flakes, or fine powder are affected very little by even prolonged contact with inert hydrocarbon solvents. Consequently, even though the pigments are soluble in mixtures of cottonseed oil and hydrocarbons, they are protected from such action because of the resistance to rupture of the glands in which they

TABLE 1

Effect of Organic Liquids on Pigment Glands

Sample No.	Previous treatment	Physical state	Solvent	Appearance after 24 hours contact
CSA 3	Ground	Powder	Petroleum ether	Practically all glands intact
CSA 3	Ground	Powder	Diethyl ether	Yellow skeletons of broken glands
C-101	None	Cross-section	Hexane	Practically all glands intact
C-101	None	Cross-section	Petroleum ether	Practically all glands intact
C-101	None	Cross-section	1,1,2-trichloroethane	Practically all glands intact
C-101	Hexane extracted	Flakes	1,1,2-trichloroethane	Practically all glands intact
C-101	None	Cross-section	1,1,2-trichloroethylene	Practically all glands intact
C-101	Hexane extracted	Flakes	1,1,2-trichloroethylene	Practically all glands intact
C-101	None	Cross-section	1,1,2,2-tetrachloroethane	Practically all glands intact
C-101	Hexane extracted	Flakes	1,1,2,2-tetrachloroethane	Practically all glands intact
C-101	None	Cross-section	1,1,2,2-tetrachloroethylene	Practically all glands intact
C-101	Hexane extracted	Flakes	1,1,2,2-tetrachloroethylene	Practically all glands intact
C-101	None	Cross-section	Benzene	Practically all glands intact
C-101	Hexane extracted	Flakes	Benzene	Practically all glands intact
C-101	None	Cross-section	Tetralin	Practically all glands intact
C-101	Hexane extracted	Flakes	Tetralin	Practically all glands intact
C-101	None	Cross-section	Mineral oil	Practically all glands intact
C-101	Hexane extracted	Flakes	Mineral oil	Practically all glands intact
C-101	None	Cross-section	1,4-dioxane	Yellow skeletons of broken glands
C-101	Hexane extracted	Flakes	1,4-dioxane	Yellow skeletons of broken glands
C-101	None	Cross-section	Acetone	Yellow skeletons of broken glands
C-101	Hexane extracted	Flakes	Acetone	Yellow skeletons of broken glands
C-101	None	Cross-section	Ethyl methyl ketone	Yellow skeletons of broken glands
C-101	Hexane extracted	Flakes	Ethyl methyl ketone	Yellow skeletons of broken glands
C-101	None	Cross-section	Diethyl ether	Yellow skeletons of broken glands
C-101	Hexane extracted	Flakes	Diethyl ether	Yellow skeletons of broken glands
C-101	None	Cross-section	Chloroform	Purple skeletons of broken glands
C-101	Hexane extracted	Flakes	Chloroform	Purple skeletons of broken glands
C-77	None	Cross-section	Petroleum ether	Practically all glands intact
C-77	None	Cross-section	Diethyl ether	Yellow skeletons of broken glands
C-77	None	Cross-section	Chloroform	Purple skeletons of broken glands
C-77	None	Flakes	Hexane	Practically all glands intact
C-77	None	Flakes	Petroleum ether	Practically all glands intact
C-77	None	Flakes	Diethyl ether	Yellow skeletons of broken glands
C-77	Hexane extracted	Flakes	Diethyl ether	Yellow skeletons of broken glands

TABLE 2

Mechanical Fractionation of Cottonseed

Sample	Sample fractionated	Liquids used for fractionation	Temperature	Density of mixture	Treatment of suspension	Length of contact	Fractions obtained
			°*C.*	*g./cc.*		*hours*	
C-101	Hexane-extracted flakes	CCl_4, cottonseed oil	24.5	1.45	Waring Blendor	1½-2	Yellow liquid; hulls; tissue with glands
C-101	Hull-free fraction from C-101	CCl_4, cottonseed oil	24.5	1.36	Waring Blendor	1½-2	Dark yellow liquid; intact glands; gland-free tissue
C-101	Hexane-extracted flakes	CCl_4, cottonseed oil	24.5	1.402	Waring Blendor	1½-2	Light yellow liquid; intact glands and tissue; gland-free tissue with hulls
C-101	Hexane-extracted flakes	CCl_4, cottonseed oil	24.5	1.378	Waring Blendor	1½-2	Dark yellow liquid; intact glands; gland-free tissue with hulls
Alb. D	Dehulled, ground, and sieved	CCl_4, mineral oil	24.5	1.378	Shaken	1½-2	Yellow liquid; intact glands; gland-free tissue
Op. 10/28	Dehulled, ground, and sieved	CCl_4, mineral oil	24.5	1.378	Shaken	1½-2	Yellow liquid; intact glands; gland-free tissue
Alb. E	Dehulled, ground, and sieved	CCl_4, mineral oil	24.5	1.378	Shaken	1½-2	Yellow liquid; intact glands; gland-free tissue
Alb. H	Dehulled, ground, and sieved	CCl_4, mineral oil	24.5	1.378	Shaken	1½-2	Yellow liquid; intact glands; gland-free tissue
Alb. I	Dehulled, ground, and sieved	CCl_4, mineral oil	24.5	1.378	Shaken, rolled on miniature ball mill	1½-2	Yellow liquid; intact glands; gland-free tissue
Op. 1/6	Dehulled, ground, and sieved	CCl_4, mineral oil	24.5	1.378	Rolled on miniature ball mill	1½-2	Yellow liquid; intact glands; gland-free tissue
C-128	Hexane-extracted flakes ground	CCl_4, mineral oil	24.5	1.378	Waring Blendor	24	Cherry red liquid; intact glands; gland-free tissue with hulls
C-128	Hexane-extracted flakes ground	CCl_4, mineral oil	24.5	1.378	Waring Blendor	24	Pink liquid; intact glands; gland-free tissue with hulls
C-128	Hexane-extracted flakes ground	CCl_4, mineral oil	24.5	1.378	Waring Blendor	1½	Pale pink liquid; intact glands; gland-free tissue with hulls
C-128	Hexane-extracted flakes ground	CCl_4, mineral oil	2.0	1.378	Waring Blendor	1½	Straw yellow liquid; intact glands; gland-free tissue with hulls
C-128	Hexane-extracted flakes ground, gland-free	CCl_4, mineral oil	24.5	1.45	Waring Blendor	2-3	Very pale yellow liquid; hulls; gland-free, hull-free tissue
C-128	Hexane-extracted flakes ground, gland-free	CCl_4, mineral oil	—29.0	1.446	Shaken	2	Very pale yellow liquid; hulls; gland-free, hull-free tissue
C-98	Dehulled and flaked	CCl_4, mineral oil	2.0	1.376	Waring Blendor	2	Yellow liquid; intact glands; gland-free tissue
C-98	Petroleum ether-extracted flakes	CCl_4, mineral oil	2.0	1.376	Waring Blendor	2	Yellow liquid; encrusted glands (intact); gland-free tissue
C-78	Dehulled and flaked	CCl_4, mineral oil	2.0	1.376	Waring Blendor	2	Yellow liquid; intact glands; gland-free tissue
C-78	Petroleum ether-extracted flakes	CCl_4, mineral oil	2.0	1.376	Waring Blendor	2	Yellow liquid; encrusted intact glands; gland-free tissue
C-78	Dehulled and flaked	CCl_4, hexane	24.5	1.378	Waring Blendor	2	Pink liquid; intact glands; gland-free tissue

occur. The crushing of a small fraction of the pigment glands during the flaking or grinding of cottonseed accounts for the small amounts of gossypol and other cottonseed pigments which are found in extracts of cottonseed obtained with the use of inert solvents such as petroleum ether (6,9,5).

The slowness with which the pigments are extracted from cottonseed even with the use of active solvents in which they are readily soluble is likewise dependent on the nature of the pigment glands. These glands are attacked only slowly by certain active solvents and the pigments can dissolve in the liquids only after the glands have been broken and the pigments exposed to the action of the solvent.

Mechanical Fractionation of Cottonseed

It was found that the density of the pigment glands differs from that of other seed and hull tissues. The density of the glands varies from 1.26 to 1.38 g./cc.; that of the tissue from 1.40 to 1.45 g./cc.; and that of the hulls is greater than 1.45 g./cc.

By utilization of the various properties of the pigment glands a process was developed for the mechanical fractionation of cottonseed into pigments, oil, and meal. In this process the stability of the pigment glands, which is responsible for many of the difficulties experienced when ordinary solvent extraction techniques are applied in processing cottonseed, has been used as the basis for mechanically separating the glands from other parts of the seed. This mechanical separation is accomplished by treating finely divided cottonseed with organic liquids which do not attack the pigment glands and which have a density intermediate between those of the various parts of the seed being separated.

The complete separation of cottonseed into pigment glands, oil, seed tissue, and hull tissue must be carried out in two successive steps with the use of two liquid mixtures as outlined below:

(a) Finely ground cottonseed is treated with a mixture of inert organic liquids of a density less than that of the hulls and greater than that of the pigment glands and other embryo tissue. The glands and ground tissue float in this medium and the ground hull fragments sink. The mixture may be centrifuged in order to pack the precipitated hulls tightly. The supernatant liquid along with the pigment glands and other cottonseed tissue is decanted.

(b) The density of the liquid mixture is now decreased by the addition of more of the lighter organic liquid so that the density of the new liquid mixture is intermediate between that of the pigment glands and the other embryo tissue. After thorough agitation the mixture is allowed to stand. The pigment glands float on the surface of the liquid and the other tissue sinks to the bottom. Centrifuging packs the precipitated tissue more tightly so that the pigment glands and liquid can more easily be decanted from the tissue. The glands are filtered to free them of liquid, and the oil is recovered from the liquid mixture (miscella) by distillation.

The process may be repeated on either the precipitated tissue (meal) or the levitated pigment glands in order to effect a further separation of glands which may have adhered to particles of tissue and so have been carried down with them, or to separate the tissue which may have adhered to the glands and so have floated up with them.

The order of separation may be reversed. The pigment glands may first be separated from other embryo tissue and hull fragments and the gland-free embryo tissue (meal) then separated from hull fragments. The second separation can be omitted in the preparation of embryo tissue if it is to be freed only of pigment glands but not of the small amount of hull fragments which usually accompany the kernels because of inadequate dehulling.

When the process is applied directly to flaked or finely ground cottonseed without preliminary defatting, recovery of the lightly pigmented oil from the solvent mixture (miscella) provides effective separation into three parts, namely, oil, pigment glands, and pigment-free seed tissue (meal). The process can also be used as a supplement to solvent extraction of cottonseed with inert hydrocarbons. By this procedure the oil is first extracted with an inert hydrocarbon solvent such as *n*-hexane, and then the pigment glands may be separated from the defatted meal by the fractionation process just described.

Because of the tenacity with which the pigment glands cling to the other seed tissue the seed must be very finely divided in order to obtain glands free of adhering tissue. This is best accomplished in the laboratory by severe agitation, *e.g.*, in a Waring Blendor, of a suspension of thin cottonseed flakes in the liquid mixture used for the flotation process. When defatted cottonseed flakes are used, they may be finely ground either before they are treated with the mixture for floating off the glands or while they are in contact with the mixture. When the defatted flakes are ground prior to fractionation with organic liquids, the glands are found to be almost completely encrusted with tissue. These glands can be freed of encrusted tissue only by severe and prolonged agitation during contact with the organic liquids used for the fractionation. The mechanical fractionation of various seed samples by the process described above with the use of various liquid mixtures is summarized in Table 2.

Properties of the Mechanically Fractionated Products

When the process for the mechanical fractionation of cottonseed is applied directly to flaked cottonseed for the recovery of oil, prolonged contact and high temperatures must be avoided in order to prevent rupture of the pigment glands. Separations carried out rapidly and at low temperatures cause the rupture of only a few pigment glands so that the recovered oil is lightly colored.

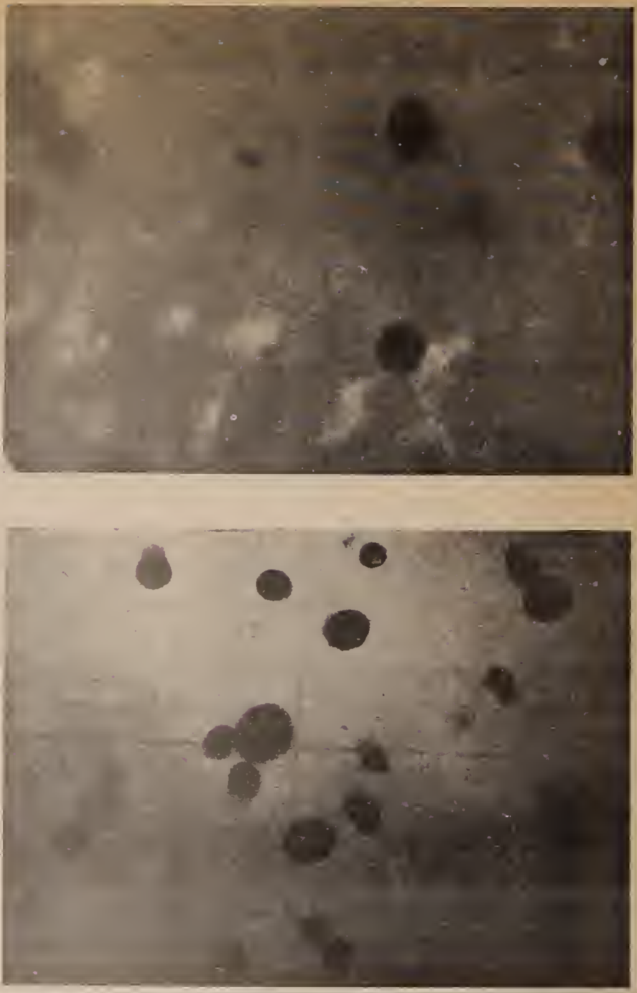

FIG. 2. Photomicrographs of cottonseed flake and separated pigment glands.

(a) Cottonseed flake; × 170.

(b) Separated pigment glands; × 63.

TABLE 3

Alkaline Extracts of Cottonseed Fractions

Sample No.	Treatment prior to fractionation	Seed fraction extracted with alkali	Color of extract	Light absorption of extracts in terms of extinction × 100 at		
				600 mμ	500 mμ	400 mμ
C-101	Hexane-extracted	Unfractionated	Brown	31.2	58.0	164.0
C-101	Diethyl ether-extracted	Unfractionated	Tan	11.4	22.0	95.0
C-101	Hexane-extracted	Gland-free, hull-free tissue	Yellow	7.0	15.7	95.0
C-128	Hexane-extracted	Unfractionated	Tan	19.9	43.5	174.8
C-128	Hexane-extracted	Gland-free tissue (fractionated at 24.5°C., 24 hours)	Yellow	9.6	25.4	127.5
C-128	Hexane-extracted	Gland-free tissue (fractionated at 2°C., 24 hours)	Yellow	13.8	31.0	119.4
C-128	Hexane-extracted	Gland-free tissue (fractionated at 24.5°C., 1½ hours)	Yellow	11.5	27.6	128.4
C-128	Hexane-extracted	Gland-free tissue (fractionated at 2°C., 1½ hours)	Yellow	13.0	29.7	131.0
C-98	Petroleum ether-extracted	Unfractionated	Tan	36.5	65.76	224.7
C-98	Petroleum ether-extracted	Gland-free tissue (fractionated at 2°C., 1½ hours)	Yellow	13.7	37.88	190.5
C-98	Dehulled, flaked	Gland-free tissue (fractionated at 2°C., 1½ hours)	Yellow	12.8	36.75	186.7
C-78	Petroleum ether-extracted	Unfractionated	Tan	21.4	48.4	250.5
C-78	Petroleum ether-extracted	Gland-free tissue (fractionated at 2°C., 1½ hours)	Yellow	29.75	25.10	125.2
C-78	Dehulled, flaked	Gland-free tissue (fractionated at 2°C., 1½ hours)	Yellow	9.15	22.9	110.2

Since the separation is purely mechanical, the intact pigment glands suffer no alteration. Even when prolonged contact at moderate temperatures produces rupturing of some of the pigment glands with a consequent dispersion of the color in the liquid, as shown in Table 2, the remaining intact separated glands do not differ from those in the original seed. The conditions during the separation affect only the quantity of the glands recovered.

As shown in the photomicrographs in Figure 2 of the separated pigment glands and a cottonseed flake before the glands were separated, the glands suffer no change during separation. An accurate comparison of the pigmentation was obtained by spectrophotometric analysis of chloroform extracts of the separated glands and of the original flaked seed. Comparison of the extracts showed that they contained the same relative amounts of gossypol (2), gossyfulvin (3), and gossypurpurin (1); and, therefore, it can be assumed that the separated glands are identical in all other respects with the glands in the original seed.

Visual examination showed that the gland-free tissue was of a lighter color than the original seed before removal of the glands, and also lighter than the hexane-extracted seed, or the diethyl ether-extracted seed. Likewise, as shown in Table 3, alkaline extracts of the gland-free tissue were less deeply colored than corresponding alkaline extracts of the original seed (4). For accurate comparison of the pigmentation the spectral absorption of alkaline extracts of the original seed and various fractionated products were determined at 20 mμ intervals from 600 to 400 mμ using a Coleman monochromator spectrophotometer. The absorption spectra curves thus obtained showed no characteristic maxima or points of inflection but were all approximately linear, and all showed increased absorption with decreasing wave length. Consequently, the relative extinctions at 600, 500, and 400 mμ listed in Table 3 were compared in order to obtain an index of the relative intensities of the color of the alkaline extracts. Comparison of these data confirm the visual observation that seed tissue (meal) which has been mechanically freed of pigment glands is lighter in color than either hexane-extracted or diethyl ether-extracted cottonseed. Analysis of the data obtained on extracts of samples of cottonseed C-78 and C-98 which were mechanically fractionated with and without prior defatting with *n*-hexane shows that the most lightly pigmented tissue (meal) is obtained by mechanical fractionation of cottonseed which has not been subjected to preliminary defatting with *n*-hexane. On the other hand, the spectrophotometric data on alkaline extracts of gland-free samples of cottonseed C-128 show that the temperature and time of contact of seed and liquid during the fractionation process do not significantly affect the color of the recovered seed tissue (meal) even though these conditions affect the number of pigment glands broken during the separation and, hence, the color of the recovered oil.

Summary

1. The efficacy of a given solvent for the extraction of the gland pigments of cottonseed has been shown to be determined not only by its solvent power for the pigments but also by its ability to attack the pigment glands and the extent to which these glands are exposed to its action, which in turn depends upon the degree of subdivision of the seed being extracted.

2. The properties of the pigment glands have been utilized for the development of a method for mechanically fractionating cottonseed into pigment glands, embryo tissue (meal), and hull tissue. The process consists in the treatment of finely divided cottonseed with mixtures of inert liquids having densities intermediate between those of the seed parts being separated.

3. The fractionation method has been shown to be applicable to the preparation of pigment glands, and pigment-free oil and meal from flaked cottonseed.

4. The method has also been shown to be applicable to the separation of pigment glands from defatted cottonseed.

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